

SMB

### Antimicrobial Composition

The present invention relates to an antimicrobial composition, its use for the improvement and/or stabilization of the keeping quality of microbially perishable products, its use as a processing aid, and microbially perishable products containing said antimicrobial composition.

Industrially processed food and feed products, cosmetics, pharmaceuticals and other products susceptible to microbial perishing must be storable for not too short a period of time in order to reach the consumer in an unperished state after shipping and distribution on the usual pathways. In addition, the consumer even expects the acquired product not to perish immediately after having been bought, but to be storable for some days or weeks, depending on the kind of product.

If not treated, most food and feed products would perish within a few days since fungi and/or bacteria could thrive without any hindrance, at most impeded by cooling, on an ideal, for them, substrate. Typical examples include the perishing of bread from molds, e.g., *Aspergillus niger*, of meat products (e.g., sausages) from enterobacteria or lactobacilli, the contamination of poultry by *Salmonellae*, and many more. Since fungi including yeasts or their spores, Gram-positive and Gram-negative bacteria are present in every place except where a sterile environment has been created by particular expensive measures, which cannot be applied industrially for economic reasons, appropriate countermeasures must be taken.

Therefore, conventionally, food and feed products, cosmetics, pharmaceuticals, paints, papers and pulps and other perishable products are rendered storable using preservatives, which appear in the Codex Alimentarius list of the Food and Agriculture Organisation (FAO/WHO Food Standard Programme) in Division 3, Food Additives, 3.37, Preservatives, as "synthetic preservatives" and are mostly employed in the form of chemical monosubstances or their combinations.

From the prior art, a wide variety of additives for preserving perishable products has been known. These include, for example, additives based on flavoring agents, alcohols, organic acids, aldehydes, phenolics and essential oils. Such compositions are described, for example, in the US Patent Specification 4,446,161, US 4,927,651, WO 94/14414, GB 172,993 and DE-OS-31 38 277, and in E. Lück (Chemische Lebensmittelkonservierung, page 1977, 1986, Springer-Verlag).

The preservatives appearing in the mentioned list are bacteriostatically and/or fungistatically active and substantially improve the keeping quality. However, they are refused by many consumers since their effects on the consumer's health are not known, and adverse effects, especially for a repeated uptake over an extended period of time, cannot be excluded. Another disadvantage is that all methods known to date are based on pH or  $a_w$  value changes.

As a solution to this problem, WO 96/29895 proposes antimicrobial compositions comprising several GRAS (generally recognized as safe) flavoring agents. On the one hand, these compositions employ only GRAS flavoring agents, which are considered safe under the food law. In addition, a synergistic antimicrobial effect could be observed due to which considerably lower amounts of the flavoring agents (preservatives) can be employed.

WO 98/58590 (published December 30, 1998) describes further antimicrobial compositions wherein mixtures of polyphenol and a GRAS flavor alcohol or of benzyl alcohol and another GRAS flavor alcohol are additionally admixed with other components, such as (a) monohydric or polyhydric alcohols containing from 2 to 10 carbon atoms, (b) organic acids containing from 1 to 15 carbon atoms or their physiologically acceptable salts, and/or (c) water-soluble solubilizers, especially glycerol or propylene glycol.

One particular disadvantage of these preservatives is their being added to the food product in high concentrations, as a rule. Thus, relatively large amounts of these substances also arrive in the human body when the food is ingested. This causes responses in the form of allergic diseases, which today are often met at a high incidence.

An alternative to the preservation by the addition of synthetic preservatives is the thermal inactivation of germs, e.g., by pasteurizing. Pasteurizing is a thermal treatment at 70 to 85 °C with an exposure time of from 30 to 120 minutes.

Pasteurization substantially improves the keeping quality of thus treated products, but is technically complicated and consumes very much energy. In addition, the viability of spores is often not or only very incompletely eliminated. Also, for thermally sensitive products, pasteurization cannot be used, or it leads to a significant loss in quality since the "degree of freshness" of the pasteurized product will be reduced, at least by the second thermal treatment (at up to 85 °C), which is often necessary. In addition, it is often just the valuable components of food products, cosmetics or pharmaceuticals, e.g., vitamins, amino acids and many pharmaceutically active ingredients, which are thermolabile, so that a thermal treatment under the usual pasteurization conditions is precluded.

Another possibility for improving the keeping quality is to place the product susceptible to perishing in an air-tight package under nitrogen or CO<sub>2</sub>, or to supply it in vacuum packages, as is done, for example, with ground coffee. However, these processes are expensive and tedious and thus, for many food products, cannot be used.

Accordingly, it has been the object of the present invention to provide an additive for the improvement of the keeping quality and/or stabilization of microbially perishable products which lacks the mentioned drawbacks of the prior art.

Surprisingly, it has been found that the antimicrobial effect of the composition of GRAS flavoring agents described in WO 96/29895 can be further enhanced when one of the components of the compositions is a hydrophilic GRAS flavoring agent.

Accordingly, the present application relates to:

- (1) an antimicrobial composition which is
  - (A) a mixture comprising at least two GRAS (generally recognized as safe) flavoring agents, except polyphenol compounds and benzyl alcohol, and at least one hydrophilic alcoholic GRAS flavoring agent and/or at least one hydrophilic non-alcoholic GRAS flavoring agent; or
  - (B) a mixture comprising benzyl alcohol or polyphenol compounds and at least one non-alcoholic hydrophilic GRAS flavoring agent, the mixture containing no other GRAS flavor alcohols;

wherein said hydrophilic alcoholic GRAS flavoring agent is a monohydric or polyhydric alcohol containing from 2 to 10 carbon atoms,

and said hydrophilic non-alcoholic GRAS flavoring agent is an organic acid containing from 1 to 15 carbon atoms or its physiologically acceptable salt, a hydrophilic acetate and/or a hydrophilic aldehyde;

- (2) a method for the improvement and/or stabilization of the keeping quality of microbially perishable products, characterized in that an antimicrobial composition as defined in (1) above is added as an additive to said microbially perishable product;
- (3) the use of the antimicrobial composition as defined in (1) above as an additive for microbially perishable products, especially as an additive for food products and cosmetics;
- (4) a method for the improvement and/or stabilization of the keeping quality of microbially perishable products in which the surfaces of the products and/or their environment, especially the ambient air and/or the surfaces of the equipment or other materials immediately contacting the products, are treated with one or more processing aids before, after or during the process for the manufacturing, processing or packaging of the products, characterized in that said processing aid comprises an antimicrobial composition as defined in (1) above;
- (5) the use of the antimicrobial composition as defined in (1) above as a processing aid; and
- (6) a microbially perishable product, especially in food products, cosmetics or pharmaceuticals, containing the antimicrobial composition as defined in (1) above.

In the following, the compositions (A) and (B) according to the invention are further described in more detail:

The GRAS flavoring agents in the mixtures (A) and (B) are recognized by the FDA authority as commercially safe for use in foods (GRAS = generally recognized as safe in food). The mentioned GRAS flavor alcohols and GRAS flavoring agents are the compounds mentioned in the FEMA/FDA GRAS Flavour Substances Lists GRAS 3-15 No. 2001-3815 (as of 1997). This list contains natural and synthetic flavoring agents approved by the American public health authority, FDA, for use in foods (FDA Regulation 21 CFR 172.515 (Synthetic Flavoring Substances and Adjuvants) and FDA Regulation 21 CFR 182.20 (Natural Flavoring Substances and Adjuvants)).

The hydrophilic alcoholic GRAS flavoring agent is preferably a monohydric or polyhydric alcohol containing from 2 to 7 carbon atoms. Especially preferred are 1-propanol (propyl alcohol), glycerol, propylene glycol, acetoin (acetylmethylcarbinol), ethanol and 2-propanol (isopropanol). However, when the antimicrobial composition is used for treating food products or as an additive in foods, it is recommendable to keep the content of ethanol or 2-propanol as low as possible or to dispense with them altogether.

The organic acid preferably contains from 2 to 10 carbon atoms. Particularly preferred are acetic acid, aconitic acid, formic acid, malic acid (hydroxy-succinic acid), lactic acid, phenylacetic acid ( $\alpha$ -toluenic acid), citric acid, mandelic acid (hydroxyphenylacetic acid), tartaric acid, fumaric acid, tannic acid, hydrocinnamic acid (3-phenyl-1-propionic acid) and their physiologically acceptable salts. The physiologically acceptable salts comprise the alkali, alkaline earth and ammonium salts.

The hydrophilic GRAS acetates are preferably allicin, triacetin (glycerol triacetate), potassium acetate, sodium acetate and calcium acetate. The hydrophilic GRAS aldehydes are preferably furfurol, propionic aldehyde (propanal) and vanillin.

The GRAS flavoring agents of mixture (A) are preferably lipophilic GRAS flavoring agents. In particular, the GRAS flavoring agents of mixture (A) are selected from the following components: (a) lipophilic alcohols, (b) phenols, (c) esters, (d) terpenes, (e) acetals, (f) lipophilic aldehydes, (g) essential oils, (h) lipophilic acids and their derivatives.

According to the invention, mixture (A) may contain one or more lipophilic GRAS flavor alcohols (a) or their derivatives. According to the invention, it is preferred to use two or three GRAS flavor alcohols. In detail, the following lipophilic GRAS flavor alcohols may be employed, for example: n-butyl alcohol (n-propyl carbinol), iso-butyl alcohol (2-methyl-1-propanol), hexyl alcohol (hexanol), L-menthol, octyl alcohol (n-octanol), cinnamyl alcohol (3-phenyl-2-propene-1-ol),  $\alpha$ -methylbenzyl alcohol (1-phenylethanol), heptyl alcohol (heptanol), n-amyl alcohol (1-pentanol), iso-amyl alcohol (3-methyl-1-butanol), anisic alcohol (4-methoxybenzyl alcohol, p-anisic alcohol), citronellol, n-decyl alcohol (n-decanol), geraniol,  $\beta$ - $\gamma$ -hexenol (3-hexenol), lauryl alcohol (dodecanol), linalool, nerolidol, nonadienol (2,6-nonadien-1-ol), nonyl alcohol (nonanol-1), rhodinol, terpineol, borneol, clineol (eucalyptol), anisole, cuminyl alcohol (cuminol), 10-undecen-1-ol, 1-hexadecanol. As said derivatives, both natural and synthetic (naturally occurring or not) derivatives can be employed. Suitable derivatives include, for example, the esters, ethers and carbonates of the above mentioned GRAS flavor alcohols. However, if said at least two GRAS flavoring agents in mixture (A) are exclusively GRAS flavor alcohols, a hydrophilic non-alcoholic GRAS flavoring agent is preferably employed.

As components (b), the following phenol compounds may be employed: thymol, methyleugenol, acetylene, safrol, eugenol, isoeugenol, anethole, phenol, methylchavicol (estragol; 3-(4-methoxyphenyl)-1-propene), carvacrol,  $\alpha$ -bisabolol, fornesol, anisole (methoxybenzene), propenylguaethol (5-propenyl-2-ethoxyphenol) and their derivatives.

Derivatives of phenol compounds according to the present invention are compounds in which the phenolic hydroxy group is esterified or etherified.

As lipophilic esters (component (c)), the following acetates may be used: iso-amyl acetate (3-methyl-1-butyl acetate), benzyl acetate, benzylphenyl acetate, n-butyl acetate, cinnamyl acetate (3-phenylpropenyl acetate), citronellyl acetate, ethyl acetate (acetic ester), eugenol acetate (acetylene), geranyl acetate, hexyl acetate (hexanyl ethanoate), hydrocinnamyl acetate (3-phenylpropyl acetate), linalyl acetate, octyl acetate, phenylethyl acetate and terpinyl acetate. Further suitable esters include ester derivatives of the above defined hydrophilic GRAS flavor acids and the lipophilic GRAS flavor acid (component (h)), for example, their C<sub>1-6</sub> alkyl esters and benzyl esters.

As terpenes (component (d)), there may be used, for example, camphor, limonene and  $\beta$ -caryophyllene.

The acetals (component (e)) which can be used include, e.g., acetal, acetaldehyde dibutyl acetal, acetaldehyde dipropyl acetal, acetaldehyde phenethyl propyl acetal, cinnamic aldehyde ethylene glycol acetal, decanal dimethyl acetal, heptanal dimethyl acetal, heptanal glyceryl acetal and benzaldehyde propylene glycol acetal.

As lipophilic aldehydes (component (f)), there may be used, e.g., acetaldehyde, anisic aldehyde, benzaldehyde, iso-butyl aldehyde (methyl-1-propanal), citral, citronellal, n-capraldehyde (n-decanal), ethylvanillin, heliotropin (piperonal), heptyl aldehyde (heptanal), hexyl aldehyde (hexanal), 2-hexenal ( $\beta$ -propylacrolein), hydrocinnamic aldehyde (3-phenyl-1-propanal), lauryl aldehyde (dodecanal), nonyl aldehyde (n-nonanal), octyl aldehyde (n-octanal), phenylacetaldehyde (1-oxo-2-



phenylethane), cinnamic aldehyde (3-phenylpropenal), perillaldehyde and cuminaldehyde.

The following essential oils and/or alcoholic or glycolic extracts or extracts obtained by a CO<sub>2</sub> high-pressure process from the mentioned plants (component (g)) can also be employed according to the invention:

(g1) oils or extracts having a high content of alcohols: melissa, coriander, cardamon, eucalyptus;

(g2) oils or extracts having a high content of aldehydes: Eucalyptus citriodora, cinnamon, lemon, lemon grass, melissa, citronella, lime, orange;

(g3) oils or extracts having a high content of phenols: origanum, thyme, rosemary, orange, clove, fennel, camphor, mandarin, anise, cascarilla, estragon and pimento;

(g4) oils or extracts having a high content of acetates: lavender;

(g5) oils or extracts having a high content of esters: mustard, onion, garlic;

(g6) oils or extracts having a high content of terpenes: pepper, bitter orange, caraway, dill, lemon, peppermint, nutmeg apple.

As lipophilic acids (component (h)), the following acids may be used: adipic acid, capronic acid, pelargonic acid (nonanoic acid), valeric acid (pentanoic acid), iso-valeric acid (3-methylbutyric acid), phenoxyacetic acid (glycolic acid phenyl ether), cinnamic acid (3-phenylpropenoic acid), their derivatives, such as amides (including N-substituted amides) and salts (alkali, alkaline earth and ammonium salts), and lipophilic derivatives of the above mentioned hydrophilic acids [e.g., their N-substituted amides and compounds modified (acylated and alkylated) at the side-chain hydroxy functions].

Mixture (A) preferably contains from 0.01 to 90% by weight, preferably from 0.1 to 50% by weight, of GRAS flavoring agents (a) to (h). It is particularly preferred for mixture (A) to contain from 0.01 to 30% by weight, preferably from 0.1 to 10% by weight, of lipophilic GRAS flavoring agents (a) to (h).

The proportion of hydrophilic alcoholic GRAS flavoring agents may be up to 99% by weight of mixture (A) and preferably is from 30 to 98% by weight, more preferably from 80 to 95% by weight. The proportion of hydrophilic non-alcoholic GRAS flavoring agents may be up to 90% by weight of mixture (A) and preferably is from 0.1 to 50% by weight.

The mixing ratio of the individual components of mixture (A) [hydrophilic alcoholic GRAS flavoring agents; hydrophilic non-alcoholic GRAS flavoring agents; components (a) to (h)] is between 10,000:1 and 1:10,000, preferably between 1000:1 and 1:1000 and more preferably between 100:1 and 1:100.

Particularly preferred as mixture (A) is a composition containing at least two of the above defined GRAS flavor alcohols (component (a)) and at least one of said hydrophilic non-alcoholic GRAS flavoring agents.

In mixture (B), lipophilic polyphenol compounds are preferably employed, especially the following polyphenols: pyrocatechol, resorcinol, hydroquinone, phloroglucinol, pyrogallol, hexahydroxybenzene, usnic acid, acylpolyphenols, lignins, anthocyanins, flavones, catechols, gallic acid derivatives (e.g., tannins, gallotannin, tannic acids, gallotannic acids), their derivatives, such as (2,5-dihydroxyphenyl)carboxylic and (2,5-dihydroxyphenyl)alkanecarboxylic substitutions, salts, esters and amides, caffeic acid and its esters and amides, flavonoids (e.g., flavone, flavonol, isoflavone, gossypetin, myricetin, robinetin, apigenin, morin, taxifolin, eriodictyol, naringin, rutin, hesperidin, troxerutin, chrysin, tangeritin, luteolin,

catechols, quercetin, fisetin, kaempferol, galangin, rotenoids, aurones, flavonols, flavonediols), extracts, e.g., from Camellia, Primula. Further, their possible derivatives, e.g., salts, acids, esters, oxides and ethers, may also be used. A particularly preferred polyphenol is tannin (a GRAS compound).

Mixture (B) preferably consists of from 0.01 to 99% by weight, preferably from 0.1 to 90% by weight, of benzyl alcohol or polyphenol compounds, and from 0.01 to 50% by weight, preferably from 0.1 to 30% by weight, of hydrophilic non-alcoholic GRAS flavoring agents. In addition, mixture (B) can contain further GRAS flavoring agents, preferably lipophilic ones, such as the above defined (b) phenols, (c) lipophilic esters, (d) terpenes, (e) acetals, (f) lipophilic aldehydes, (g) essential oils and (h) lipophilic acids, and their derivatives.

Preferably, phenols (b) and/or essential oils (g) are used as said further GRAS flavoring agents.

The proportion of the further components (b) to (h) in mixture (B) may be up to 50% by weight, but preferably is within a range of from 0.001 to 25% by weight, and more preferably within a range of from 0.01 to 9% by weight. The mixing ratio of the individual components of mixture (B) [benzyl alcohol or polyphenol compounds; non-alcoholic GRAS flavoring agents; components (b) to (h)] is between 10,000:1 and 1:10,000, preferably between 1000:1 and 1:1000, more preferably between 100:1 and 1:100.

Particularly preferred according to the present invention are antimicrobial compositions in which the antimicrobially active ingredient exclusively consists of GRAS flavoring agents, i.e., does not contain any "derivatives" of the GRAS flavoring agents. This is particularly important when the antimicrobial composition is intended to contact food products.

In another embodiment, the composition may further contain emulsifiers, stabilizers, antioxidants, preservatives, solvents and/or carriers.

The increased antimicrobial activity of mixtures (A) and (B) relies on the fact that most of the flavoring agents are exclusively fat-soluble (lipophilic). For use in the food products field, in particular, it is nevertheless required that synergisms with hydrophilic flavoring agents exist since the lipophilic flavoring agents can otherwise display their microbicidal activity only insufficiently in food products and raw materials, which mostly predominantly contain water.

Thus, it is necessary that at least one flavoring agent is additionally hydrophilic in order to include other flavoring agents, which may be lipophilic, in synergisms as solubilizers to display a microbicidal effect in common at, in or on food products and/or raw materials.

On the other hand, hydrophilic compounds, such as GRAS flavor acids, alone only dissolve in exclusively water-containing food products. Since food products and raw materials mostly also contain fat, it is necessary that a composition of flavoring agents have lipophilic properties.

The antimicrobial activity of the composition according to the invention relies on the following new principle of action: The composition permits penetration of its components into the microorganism and thus prevents its reproduction, but without destroying it, as is done, for example, by preservatives or ethanol as single substances in terms of coagulation (denaturation) of the protein in the microorganism.

Thus, the antimicrobial compositions according to the invention are useful as additives for the improvement and/or stabilization of the keeping quality of microbially perishable products, such as food and feed products, pharma-

ceuticals and cosmetics. In particular, they are useful as additives for the following groups of foods:

Breads, pastries, improvers, baking powders, blanchmange powders, beverages, dietetic food products, essences, delicatessen, fish and fish products, potatoes and products based on potatoes, spices, flours and meals, margarine, fruits and vegetables and products based on fruits and vegetables, pickled preserves, starch products, sweets, soups, pasta food, meat and meat products, milk, dairy and cheese products, poultry and poultry products, oils, fats and products containing oils or fats.

The additives according to the invention are effective, in particular, against molds, yeasts and bacteria (Gram-positive and Gram-negative). They are excellently effective, in particular, against pathogens (Enterobacteriaceae, e.g., *E. coli*, *Salmonella*, *Enterococci*, e.g., *Staphylococci*, *Streptococci*, *Listeria*) and also against perishing-causing agents, such as *Aspergillus niger*, yeasts, e.g., *Endomyces tibuliger*. Also, the additives according to the invention act on viruses and have a reductive effect against microbial toxins, such as aflatoxins, enterotoxins.

The additives are preferably added to the microbially perishable product in amounts of from 1 ppm to 10% by weight, preferably from 1 ppm to 1.0% by weight. Particularly preferred amounts are from 0.001% by weight to 0.5% by weight. Especially preferred amounts are from 0.002% by weight to 0.25% by weight.

According to the invention, it is surprising that the effect of the additives according to the invention can be seen when concentrations as low as those mentioned are used. This is all the more surprising since the food products treated with the additives according to the invention have a significantly higher keeping quality than the perishable products treated with conventional preservatives.

It is also surprising that the advantages described are seen even for microbial exposure times of less than 24 h, especially less than 60 minutes, preferably from 1 to 60 minutes, most preferably from 5 to 15 minutes.

Surprisingly, the additives according to the invention do not result in any disadvantages in the taste, smell or color of the treated food product. One particular advantage over the prior art is that no shifts of the pH or  $a_w$  value is observed, i.e., the activity of the additives employed is surprisingly independent of the pH and  $a_w$  values. It is also surprising that the additives can be used irrespective of humidity, fat, protein and carbohydrate contents. Finally, the combinations according to the invention are insensitive to temperature variations within a range of from -30 °C to 200 °C, i.e., they are both cold and heat resistant.

The additives may also be added to the microbially perishable products in the form of sustained release formulations. A suitable sustained release formulation involves, for example, the microencapsulation of the antimicrobial composition. As the microencapsulation material, maltodextrose or a cellulose derivative can be used.

In addition, the antimicrobial composition according to the invention may also be used as a processing aid for the processing of the above defined microbially perishable products. This means that the surfaces of the products and/or their environment, especially the ambient air and/or the surfaces of the equipment or other materials immediately or mediately contacting the products, are treated with one or more processing aids prior to, during or after completion of the process for the manufacturing, processing or packaging of the products.

"Treating" within the meaning of the present invention comprises the following processing operations: brushing, spreading, emulsifying, separat-

ing, cleaning, spraying, nebulizing, volatilizing, cutting, immersing and marinating.

As a processing aid, the antimicrobial composition is used neat, in a water-soluble dilution with water, in solvents acceptable for foods (e.g., alcohols) or in fat-soluble dilutions with vegetable fats or oils.

Preferred is the use of the processing aids for production in food and feed products, cosmetics, pharmaceuticals, paints, papers and/or pulps.

In particularly preferred embodiments, the processing aids are used for the improvement and stabilization of the keeping quality of food products selected from the following group:

Breads, pastries, improvers, baking powders, blancmange powders, beverages, dietetic food products, essences, delicatessen, fish and fish products, potatoes and products based on potatoes, spices, flours and meals, margarine, fruits and vegetables and products based on fruits and vegetables, pickled preserves, starch products, sweets, soups, pasta food, meat and meat products, milk, dairy and cheese products, poultry and poultry products, oils, fats and products containing oils or fats.

The processing aid displays its activity in the environment of the perishable product, for example, a food or feed product, e.g., on machine parts which contact the product to be worked or processed, or in the air. Due to the direct contact with the surface of the perishable product, they display their activity there as well, i.e., they display their activity on the surface or, when penetrating the product, within the product itself.

Therefore, a particular advantage of the processing aid described is that it is a reliable decontaminant, on the one hand, its effectiveness against Gram-positive and Gram-negative bacteria, fungi including yeasts and also viruses

having been proven, while on the other hand, it is no danger to the consumer of the food since it is absolutely harmless towards him and has no microbicidal technological after-effect in the food, because its microbicidal activity relates to the production environment which is freed from contaminating microorganisms by the measures according to the invention.

It is particularly preferred that the microbially perishable products are simultaneously treated by the addition of additives and by external treatment with processing aids.

As mentioned above, the flavoring agents contained in the processing aids are exclusively natural or synthetic (but identical with naturally occurring) flavoring agents which have been recognized as safe according to the FEMA (GRAS - generally recognized as safe). The flavoring agents which meet these FDA standards may be used "quantum satis", i.e., they may be contained in the food up to the maximum concentration in which they do not cause any adverse effects on the smell or taste of the food to which they are added. The flavoring agents listed according to the FEMA are largely identical with the substances contained in the corresponding European standard COE.

In addition, the flavoring agents classified as "NAT4" according to Article V European Community Directive Flavourings (June 22, 1988) may also be used according to the invention, provided they are considered safe according to the above mentioned FEMA GRAS list. NAT4 substances are substances which are to be declared as "synthetic, but identical with naturally occurring substances" under certain circumstances, e.g., if these substances are used in connection with and as a component of a natural or synthetic (but identical with a naturally occurring) flavoring agent.

A particular advantage of the processing aids is the fact that they may be added to foods without hesitation in a "quantum satis" concentration range



due to their components which are listed in the FEMA GRAS list and have been recognized as safe by the US public health authority FDA, which is perhaps the most critical public health authority of all.

Another particular advantage is that the processing aids do not affect the smell and taste of the treated products.

The processing aids according to the invention are employed, for example, in the form of lubricants, emulsifiers and cleaning agents, spraying media, nebulizing media, gas-phase active agents, heat transfer media, and cutting media. The processing aids may also be employed as additives to the agents mentioned. For more details relating to the use as processing aids, reference is made to WO 96/29895 which is included herein by reference in its entirety.

It is essential that the processing aids are not added to or mixed with the microbially perishable products (food products). Rather, only the surfaces or cuts of the food products are treated with the processing aids. This may be done by directly treating the surfaces or cuts of the food products with the processing aids. However, it is also possible to add the processing aids to the surfaces of equipment, production machines, packaging means, conveying means, packaging materials and the ambient air.

It is surprising that the microbicidal activity of the processing aids can be seen already when low concentrations are used. When applied to food products, only from 0.01 to 5 g/kg, preferably from 0.05 to 2 g/kg, more preferably from 0.05 to 1 g/kg of food is used. When used for the ambient air, for example, only from 0.001 to 10 g/m<sup>3</sup> of air is used. For the surfaces of equipment, even as little as from 0.000001 g to 0.1 g/cm<sup>2</sup> surface area is used.

If these concentrations are adhered to, the quantities detectable in the food products are only around 0.001% by weight. In contrast, according to the prior art, from 0.1 to 3% by weight of preservative will be present in the food products as a rule. It is surprising that, in spite of these extremely low concentrations, an increase in storage life of up to 50% can be achieved according to the invention as compared to conventionally preserved foods.

It is to be pointed out particularly and astonishing that as little as 0.001% by weight of processing aids which are indirectly applied to food products is sufficient to achieve a stabilization or improvement of the keeping quality while the product quality is increased.

This effect is all the more surprising since the duration of the microbicidal action of the flavoring agents used according to the invention is less than 24 hours, preferably less than 12 hours. It is particularly preferred to select such processing aids and concentrations that the duration of microbicidal action is less than 1 hour, preferably less than 15 minutes.

In contrast, conventional preservatives are supposed to be active in the food product as long as possible, i.e., for weeks and months. In spite of the very short duration of action of the processing aids used according to the invention (exposure time), the keeping quality or storage life is significantly increased as compared to food products treated with conventional preservatives or preservation methods according to the prior art. Consequently, according to the invention, when the above described additives and the processing aid are combined, it is possible, surprisingly, to work with significantly lower amounts than that required when the preservatives which have been usual in the prior art are employed.

In the following, the invention will be further described by Examples:

## Bacteriological assay methods for additives

- Microorganisms: aerobic microorganisms (total germ count), Enterobacteriaceae, Enterococci, Lactobacilli, yeasts, molds. These methods are capable of determining the effects of the additives as a function of dosage and duration of action using different microorganisms on different nutrient media.

Suspension, depending on the germ being tested:

Tryptone soybean broth

wort broth

Nutrient agar: total aerobics < plate count agar  
(casein peptone glucose yeast extract agar)

Chromcult < *Enterococcus faecalis*  
*Enterococcus faecium*  
*Streptococcus bovis*

OGYE selective nutrient medium (yeast extract - glucose - oxytetracyclin)

Microorganisms (molds): *Aspergillus niger*, *Saccharomyces*

Deoxycholate lactose agar

Microorganisms

Lactose-positive - *Escherichia coli*

Lactose slightly positive - *Enterobacter* (cloacae)

Lactose slightly positive - *Klebsiella* (pneumoniae)

Lactose-negative - *Salmonella* (typhimurium and enteritidis), *Shigella* (flexneri), *Proteus* (mirabilis), *Pseudomonas*, *Enterococcus* (faecalis).

MRS-AGAR (*Lactobacillus*)

*Lactobacillus vulgaris*

Baird-Parker agar (with egg yolk-tellurite emulsion)

Microorganisms: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus* (*Enterococcus faecium*), *Bacillus subtilis*, yeasts: *Endomyces tibuliger*.

Cereus Mossel's selective agar (with egg yolk emulsion)

Microorganisms: *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus aureus*.

Deoxycholate lactose agar

Microorganisms

Lactose-positive - *Escherichia coli*

Lactose slightly positive - *Enterobacter* (cloacae), *Klebsiella* (pneumoniae).

Lactose-negative - *Salmonella* (typhimurium and enteritidis), *Shigella* (flexneri), *Proteus* (mirabilis), *Pseudomonas* (*Enterococcus faecalis*).

TGE agar (casein peptone-glucose-meat extract agar)

Microorganisms: Staphylococcus aureus, Streptococcus agalactiae, Enterococcus faecalis, Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa, Bacillus cereus.

Suspension assay - quantitative germ carrier test

Others: special nutrient media and differentiations  
for: Clostridia, Listeria and others

Contamination of the germ carriers

The contamination of the germ carriers is effected after placing in a sterile glass dish by pouring the test germ suspension ( $\geq 10^6$ /ml) over them. After 1-10 min of storing, the germ carriers are distributed in a glass dish lined with sterile filter paper, and dried in an incubator at  $36^\circ\text{C} \pm 1^\circ\text{C}$ .

Test

The contaminated and dried germ carriers are placed in sterile glass dishes and soaked with (gr. %/formulation) each; stored for 1 h and placed for the respectively designated agar/test germ and incubated in the incubator at the prescribed temperature.

After the recommended incubation time, the germ carriers are diluted in a 9-fold dilution (depending on the test germ) of  $10^1$  to  $10^8$ , and introduced in the respectively designated agar by a plate casting method.

### Calculation

All agar plates having from to 200 colonies are taken into account. The number of colony-forming units is determined using the weighted arithmetic mean:

$$C = \frac{\Sigma c}{n_1 \times 1 + n_2 \times 0,1} \times d$$

C = number of colony-forming units per ml/g

$\Sigma c$  = sum of the colonies from all Petri dishes used for the calculation

$n_1$  = number of Petri dishes of the lowest dilution stage used for the calculation ( $n_1 = 2$  for 2 Petri dishes)

$n_2$  = number of Petri dishes of the next higher dilution stage used for the calculation

d = factor of the lowest evaluated dilution stage, the dilution stage related to  $n_1$ .

### Quantitative suspension assay II - suspension test

- a) Inoculate test germ suspension with the desired test germ, e.g.,  $10^6$ /ml, expose for 1-60 min. Place desired formulation to be tested into designated germ suspension tubes (different percentages). Await end of exposure times and pour or inoculate into the appropriate agar plates, depending on the germ.

- b) Treat the test germ suspension with the desired formulation to be tested (see a) prior to inoculating the test germs (see a). Await end of exposure times and then inoculate with the respective test germs, and inoculate or pour into the appropriate agar plates depending on the test germ.

Quantitative suspension assay III - agar diffusion test

Cast nutrient agar plates which contain, e.g.,  $10^4$  microorganisms/ml.

A sterile filter paper slip (10 mm) is soaked with the formulation to be tested and placed on the nutrient agar plate.

After the incubation of (time/temperature depending on the germ), the formation of an inhibition halo is read as a positive response.

Formulation Examples

23. 3 parts of benzyl alcohol, 7 parts of lactic acid	24. 5 parts of cinnamic acid 5 parts of lactic acid 5 parts of benzyl alcohol 85 parts of soybean oil	Invention - Examples -	Formulation
$10^3$ $10^3$ $10^3$	$10^3$ $10^3$ $10^2$	5 min exp. time 15 min exp. time 60 min exp. time	total germ count
		$10^8$ /ml	control
$10^3$ $10^3$ $10^3$	$10^3$ $10^2$ $10^2$	5 min exp. time 15 min exp. time 60 min exp. time	Enterobacteria
		$10^8$ /ml	control
$10^3$ $10^3$ $10^2$	$10^4$ $10^3$ $10^3$	5 min exp. time 15 min exp. time 60 min exp. time	Enterococci
		$10^8$ /ml	control
$10^3$ $10^2$ $10^2$	$10^2$ $10^2$ $10^2$	5 min exp. time 15 min exp. time 60 min exp. time	Lactobacilli
		$10^5$ /ml	control
$10^3$ $10^2$ $10^2$	$10^2$ $10^2$ $10^2$	5 min exp. time 15 min exp. time 60 min exp. time	yeasts
		$10^5$ /ml	control
$10^2$ $10^3$ $10^4$	$10^2$ $10^2$ $10^2$	5 min exp. time 15 min exp. time 60 min exp. time	molds
		$10^5$ /ml	control



25. 1 part of essential oil a) 1 part of essential oil b) 300 parts of lactic acid 698 parts of alcohol (propylene glycol)	26. 97.9 parts of alcohol (propylene glycol) 2 parts of acid (lactic acid) 0.1 part of essential oil c)	Invention - Examples -	Formulation
10 <sup>2</sup> 10 <sup>2</sup> 10 <sup>2</sup>	10 <sup>3</sup> 10 <sup>3</sup> 10 <sup>3</sup>	5 min exp. time 15 min exp. time 60 min exp. time	total germ count
		10 <sup>8</sup> /ml	control
10 <sup>2</sup> 10 <sup>2</sup> 10 <sup>2</sup>	10 <sup>2</sup> 10 <sup>3</sup> 10 <sup>3</sup>	5 min exp. time 15 min exp. time 60 min exp. time	Enterobacteria
		10 <sup>8</sup> /ml	control
10 <sup>2</sup> 10 <sup>2</sup> 10 <sup>2</sup>	10 <sup>3</sup> 10 <sup>3</sup> 10 <sup>3</sup>	5 min exp. time 15 min exp. time 60 min exp. time	Enterococci
		10 <sup>8</sup> /ml	control
10 <sup>2</sup> 10 <sup>2</sup> 10 <sup>2</sup>	10 <sup>2</sup> 10 <sup>2</sup> 10 <sup>3</sup>	5 min exp. time 15 min exp. time 60 min exp. time	Lactobacilli
		10 <sup>5</sup> /ml	control
10 <sup>2</sup> 10 <sup>2</sup> 10 <sup>2</sup>	10 <sup>3</sup> 10 <sup>3</sup> 10 <sup>3</sup>	5 min exp. time 15 min exp. time 60 min exp. time	yeasts
		10 <sup>5</sup> /ml	control
10 <sup>3</sup> 10 <sup>2</sup> 10 <sup>2</sup>	10 <sup>3</sup> 10 <sup>3</sup> 10 <sup>3</sup>	5 min exp. time 15 min exp. time 60 min exp. time	molds
		10 <sup>5</sup> /ml	control

Example 2

Effectiveness test (quantitative suspension assay)

The effectiveness of additional mixtures according to the invention was determined according to the quantitative suspension assay described in Example 1. The results are summarized in the following Table.

	% by weight	exposure time 1 h	Staph. aureus	Asp. niger
		Reduction factor		
Propylene glycol	90%		4.9	4.0
Glycerol	9%			
anise	1%			
Propylene glycol	90%		6.5	4.0
Lactic acid	9.9%			
anise	0.1%			
Propylene glycol*			0	0
Glycerol*			0	0
Growth control			7.1	5.0

\* Comparative Example